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Pharmacokinetic-Pharmacodynamic Modeling of Unboosted Atazanavir in a Cohort of Stable HIV-Infected Patients

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Limited data on the pharmacokinetics and pharmacodynamics (PK/PD) of unboosted atazanavir (uATV) in treatment-experienced patients are available. The aim of this work was to study the PK/PD of unboosted atazanavir in a cohort of HIV-infected patients. Data were available for 58 HIV-infected patients (69 uATV-based regimens). Atazanavir concentrations were analyzed by using a population approach, and the relationship between atazanavir PK and clinical outcome was examined using logistic regression. The final PK model was a linear one-compartment model with a mixture absorption model to account for two subgroups of absorbers. The mean (interindividual variability) of population PK parameters were as follows: clearance, 13.4 liters/h (40.7%), volume of distribution, 71.1 liters (29.7%), and fraction of regular absorbers, 0.49. Seven subjects experienced virological failure after switch to uATV. All of them were identified as low absorbers in the PK modeling. The absorption rate constant (0.38 ± 0.20 versus 0.75 ± 0.28 h^{-1}; $P = 0.002$) and ATV exposure (area under the concentration-time curve from 0 to 24 h [AUC_{0-24}], 10.3 ± 2.1 versus 22.4 ± 11.2 mg · h · liter^{-1}; $P = 0.001$) were significantly lower in patients with virological failure than in patients without failure. In the logistic regression analysis, both the absorption rate constant and ATV trough concentration significantly influenced the probability of virological failure. A significant relationship between ATV pharmacokinetics and virological response was observed in a cohort of HIV patients who were administered unboosted atazanavir. This study also suggests that twice-daily administration of uATV may optimize drug therapy.

Current international guidelines for the antiretroviral treatment of adult human immunodeficiency virus (HIV) infection recommend a combined initial regimen usually based on three antiretroviral drugs (1). The combination of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a ritonavir-boosted protease inhibitor (PI) is one of the first-line options, and atazanavir (ATV) is one of the two recommended PIs in such a regimen. Atazanavir with ritonavir boosting is the only PI for which noninferiority to efavirenz-based regimens has been demonstrated in a randomized trial (1, 2). The recommended dose of ATV in adults is 300 mg, with 100 mg of ritonavir, once daily.

However, poor tolerance and drug-drug interactions, due to ritonavir in part, may complicate ritonavir-boosted atazanavir therapy in some patients. Unboosted atazanavir (uATV) may be an attractive option for addressing those issues (3). It has been shown that uATV-based therapy is noninferior to ritonavir-boosted therapy in adult treatment-naïve patients in controlling HIV infection after an initial induction treatment with ritonavir-boosted ATV (4, 5). Compared with ritonavir-boosted therapy, unboosted ATV therapy is associated with significantly lower ATV trough concentrations as well as overall exposure (6, 7). Unboosted ATV has been approved by the FDA as a 400-mg once-daily regimen, and atazanavir is currently the only PI that may be administered unboosted.

Although unboosted atazanavir is not approved by the European Medicines Agency, large-cohort studies have indicated that a significant proportion of patients do receive unboosted ATV in Europe (8–10), notably as a simplification strategy in treatment-experienced HIV-infected patients. Those cohort studies have confirmed that, in patients with stable virological suppression and no history of virological failure, the switch to unboosted atazanavir has a good efficacy and safety profile.

Early phase II data from the ATV manufacturer have demonstrated a relationship between ATV exposure and both efficacy (log drop in viral load) and safety (hyperbilirubinemia) (11). However, the "real-life" cohort studies cited above did not explore relationships between ATV exposure and outcome. Actually, limited data on the pharmacokinetics and pharmacodynamics (PK/PD) of unboosted atazanavir in treatment-experienced HIV-infected patients are available. The objective of this study was to perform PK/PD modeling of atazanavir in a cohort of stable HIV-infected patients who were administered uATV.

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MATERIALS AND METHODS

Study design, patient population, and data collection. This study was a retrospective analysis of data from a prospective observational cohort of HIV-infected patients followed in the Lyon area (France) called the Lyon HIV Cohort Study (3,200 patients are currently being followed). All data used in this study were collected during routine patient care between 2004 and 2009. Inclusion criteria for the analysis were as follows: undetectable viral load before introduction of uATV; administration of uATV for at least 1 month, whatever the backbone; and at least one ATV plasma concentration measured at the steady state. Patients who were administered uATV under the same dosage regimen for at least 2 weeks, either once daily or twice daily, were considered to be at steady state. All individuals from the cohort who met the inclusion criteria were included in the analysis. Subjects who were administered more than one uATV-based regimen (for example, because of a change in companion anti-HIV drugs or ATV dose) were included as independent individuals.

Information on atazanavir dosage regimens, blood sampling times, and ATV plasma concentrations was retrieved from the patients' electronic and manuscript medical files. Demographic data collected and tested in the model building included age, sex, and total body weight. Glomerular filtration rate (GFR) was estimated by the abbreviated Modification of Diet in Renal Disease (MDRD) equation (12). Renal function was also tested as a categorical variable in the covariate model building. Patients with an estimated GFR of ≤60 ml/min were considered as having impaired renal function. All medications that were coadministered to patients receiving uATV, including the other antiretroviral drugs, were recorded. Documented drug-drug interactions (DDI) were identified and classified in two groups in the covariate analysis: DDI that may decrease ATV exposure (e.g., tenofovir, proton-pump inhibitors, and H2 receptor antagonists) and DDI that may increase ATV exposure (e.g., triazole antifungal agents). The dosage frequency was also examined as a covariate in both pharmacokinetic and pharmacodynamic model building.

Virological outcome was monitored after the switch to uATV. Virological failure was defined as two consecutive HIV loads of >40 copies/ml in patients with undetectable viral loads before the switch to uATV.

Atazanavir assay. Atazanavir plasma concentrations were measured by a validated reverse-phase high-performance liquid chromatography (HPLC) method using an UV-diode array detector. Concentrations were determined at three wavelengths (216, 248, and 300 nm) to assess the purity of chromatographic peaks of ATV. The method was linear between 0.05 and 10 mg/liter. Interday coefficients of variation (CV) ranged from 5% for atazanavir concentrations of >0.80 mg/liter to 9% for a concentration of 0.15 mg/liter. The lower limit of quantification (LOQ) of the assay was 0.02 mg/liter. Atazanavir trough concentrations below the limit of quantification were fixed at LOQ/2 in the modeling process, which is one of the methods suggested by Beal (13).

Population pharmacokinetic modeling. A population approach was used to analyze atazanavir plasma concentrations. Nonlinear mixed effects modeling implemented in NONMEM VII software (14) (Icon Development Solutions, Ellicott City, MD) was used to identify the structural pharmacokinetic model and significant covariates that best described the data and to estimate population PK parameters, as well as interindividual and residual variability. Composite methods based on modern algorithms was performed within the NONMEM estimation procedure: for each run, the stochastic approximation expectation maximization algorithm (SAEM) was first used, and results of the SAEM estimation were then used as initial parameters in the Monte Carlo Importance sampling method (14). Analysis and postprocessing of NONMEM results were performed using the R package for NONMEM (RFN, version 2008a; Saik Urien, Paris, France) and the Matlab software (version 2011b; The MathWorks, Natick, MA).

We assumed log-normal distribution of the random parameters. An exponential error model was used to describe residual variability. Various structural and absorption models were investigated. Once the best base model had been identified, the influence of each available covariate on PK parameters was investigated. The likelihood-derived objective function calculated by NONMEM was used to assess goodness-of-fit of candidate models. In addition, mean error of prediction and mean absolute error of prediction were used to assess predictive performance. Plots of population predictions versus observed concentrations, individual predictions versus observed concentrations and residuals were also examined as graphical model diagnostics. In addition, a visual predictive check (16) was performed, by simulating 1,000 replicates based on the final model and corresponding parameters (including interindividual and residual variability) and examining the agreement between model-based simulated data and observed ATV concentrations.

Individual PK parameters of the final model, obtained by Bayesian estimation in NONMEM, were then imported into the Matlab software to simulate the time course of ATV plasma concentrations of each patient at the steady state and calculate various indexes of drug exposure, including the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}), AUC above the target concentration of 0.15 mg/liter (AUC_{target}), daily time during which ATV concentration remains above the target concentration of 0.15 mg/liter (T_{target}), and the predicted steady-state trough concentration (C_{trough}). For patients who received twice-daily uATV, a 24 h-profile with two doses administered at 0 and 12 h was simulated to calculate AUC_{0-24}, AUC_{target}, and T_{target}. The selection of the target C_{trough} of 0.15 mg/liter was based on a previous PK/PD study (17). Predicted rather than observed ATV C_{trough} values were used in the subsequent pharmacodynamic analysis, because predicted values were available for all subjects, while a few observed values were missing ($n = 3$) or reported as below the quantification limit ($n = 4$). In addition, individual predicted concentrations correlated well with observed concentrations (see Results).

Pharmacodynamic analysis. First, individual pharmacokinetic parameters of the final model, indexes of drug exposure, and uATV dosage frequencies were statistically compared between patients who experienced virological failure and those without failure. The Mann-Whitney-Wilcoxon nonparametric test was used for continuous variables, while the Fisher's exact was used for ordinal variables. Because multiple comparisons were performed, the statistical significance was fixed at 0.005 at this step.

Next, we explored the relationship between patients' exposure to uATV and virological outcome after the switch. Univariate and multivariate logistic regression were performed using the Statview software (version 5.0; SAS Institute Inc., Cary, NC) to identify pharmacokinetic variables influencing the probability of virological failure. The influence of each variable on the probability of virological failure was assessed by the likelihood ratio test (18). In this test, the difference (D) is assumed to follow a chi-squared distribution: $D = 2(\log L_{alt} - \log L_{base})$, where L is the likelihood, $\log L_{alt}$ is the log likelihood of the alternative (augmented) model, and $\log L_{base}$ is the log likelihood of the base model. Statistical significance was set at 0.05 with the appropriate degrees of freedom. Variables that were significant in the univariate analysis were then tested in a multivariate model. The final model was identified, by forward addition, again using the likelihood ratio test as the goodness-of-fit criterion. Statistical significance was set at 0.01 in the final model selection. As a limited number of failure events were observed in this study, lower-than-usual statistical significance levels were selected in order to control the number of events per variable. It has been shown that a low number of events per variable may negatively affect the estimation of the regression coefficients in logistic regression analysis (19).

RESULTS

Characteristics of the study population. Fifty-eight patients were included in the study. Among those, eight patients received two different uATV-based regimens and one received four different uATV-based regimens. As those patients met the inclusion criteria for each drug regimen that they received, a total of 69 uATV-based regimens were available for the PK/PD analysis.

TABLE 1 Demographic and treatment characteristics of patients included in the study[a]

Characteristic	No failure ($n = 62$)	Virological failure ($n = 7$)	Total ($n = 69$)
Age (yr)	50 ± 9.7	47 ± 9.4	49 ± 9.6
Sex ratio male/female (% male)	46/16 (74)	4/3 (57)	50/19 (72)
Body wt (kg)[b]	69 ± 12	70 ± 13	69 ± 12
Glomerular filtration rate (ml/min/1.73 m^2)[c]	93 ± 25	84 ± 23	92 ± 24
Renal impairment[d]	1	3	4
Dosing frequency, once daily/twice daily (% once daily administration)	21/41 (34)	6/1 (86)	27/42 (39)
Drug-drug interactions			
Increasing ATV exposure	1 (1.6)	1 (14)	2 (3)
Decreasing ATV exposure	28 (45)	3 (43)	31 (45)
Time since HIV diagnosis (yr)	11 ± 6	11 ± 5	11 ± 6
Time since first antiretroviral treatment (yr)	8 ± 5	6 ± 4	8 ± 5
Antiretroviral companion drugs			
≥2 NRTIs	57 (92)	7 (100)	64 (93)
InSTI + NRTIs	5 (8)	0 (0)	5 (7)

[a] Abbreviations: NRTI, nucleoside (or nucleotide) reverse transcriptase inhibitor; InSTI, integrase strand transfer inhibitor. Data are given as means ± standard deviations unless otherwise indicated.
[b] Initial body weight at the start of treatment.
[c] Initial GFR estimated by the abbreviated MDRD equation (data available for 61 subjects, 5 with failure and 56 without failure).
[d] Defined as estimated GFR ≤ 60 ml/min.

Demographic and treatment characteristics of the study population are presented in Table 1. In 25 cases, uATV was introduced as a switch from ritonavir-boosted ATV. The mean duration of the 69 uATV-based treatments was 16 ± 14 months (minimum, 1; maximum, 74). The various uATV dosage regimens were as follows: 300 mg once daily ($n = 4$), 400 mg once daily ($n = 22$), 600 mg once daily ($n = 1$), 200 mg twice daily ($n = 39$), and 300 mg twice daily ($n = 3$).

Seven patients experienced virological failure during their uATV-based regimen. Overall, their demographic and treatment characteristics were similar to those of patient without failure, except the dosing schedule. A 10-fold failure rate was observed in patients receiving once-daily uATV (6/27, 22%) compared with patients who received twice-daily uATV (1/42, 2.4%). This point is further discussed in the pharmacodynamic analysis.

Population pharmacokinetic modeling. One hundred twenty-eight ATV concentrations were available for the pharmacokinetic analysis (mean, 1.86 measured concentrations per patient). Data included 66 trough (predose) concentrations, 43 concentrations measured 3 h after the dose, and 19 concentrations measured at another time point.

A one-compartment model with linear elimination, fixed absorption lag-time, and mixture absorption best described ATV concentrations. Population parameter values of the final PK model are shown in Table 2. The absorption lag-time was fixed at 0.9 h, in accordance with the result from a population PK study with rich data (20). As a consequence of the mixture model of absorption, two sets of population values are provided for F and k_a. The other pharmacokinetic parameters of the model were ATV plasma clearance (CL), volume of distribution (V), proportion of regular absorbers, and the regression coefficient for the influence of body weight on ATV clearance (θ_{BW}).

For the mixture model of drug absorption, we assumed that there were two subpopulations of absorbers in the study population, namely, regular and low absorbers. Regular absorbers had a mean oral bioavailability (F) and an absorption rate constant (k_a) both fixed at 1. Low absorbers had mean values of parameters F and k_a both fixed at 0.4. Population means of F and k_a were fixed because concentration data were insufficient to estimate them properly. The initial absorption parameter values for regular and low absorbers were selected from previous works (11, 21). Various combinations of fixed parameter values were tested for the low-absorber group, and those indicated above provided the best fit. The addition of the mixture model of ATV absorption was associated with a 27-point decrease in NONMEM objective function value. Body weight was the only covariate that significantly influenced ATV plasma clearance.

Figure 1 shows observed ATV concentrations versus model predictions based on population parameter values and individual Bayesian posterior parameter values. Model-based predictions correlated well with observed ATV concentrations, with regres-

TABLE 2 Pharmacokinetic parameters of the final model estimated by NONMEM

Parameter	Population typical value (RSE, %)	Interindividual variability[a] (RSE, %)
CL (liters/h)	13.4 (8.4)	24.9 (40.7)
θ_{BW} (liters · h^{-1} · kg^{-1})[b]	0.00936 (41.3)	
V (liters)	71.1 (12.0)	47.0 (29.7)
Absorption lag time (h)	0.9 (fixed)	
$k_{a\ reg}$ (h^{-1})	1.0 (fixed)	50 (fixed)
$k_{a\ low}$ (h^{-1})	0.4 (fixed)	68.7 (89.6)
F_{reg}	1.0 (fixed)	
F_{low}	0.4 (fixed)	
Proportion of regular absorbers	0.49 (21.6)	

[a] Interindividual variability is expressed as percent coefficient of variation (CV). No interindividual variability was set for the absorption lag time or the bioavailability parameters F_{reg} and F_{low}.
[b] The influence of body weight (BW) on the typical value of ATV mean clearance (μ_{CL}) was calculated as follows: $\mu_{CL} = \theta_1 \cdot (1 + \theta_{BW} \cdot (BW-67))$, where 67 kg was the median body weight in the study population.

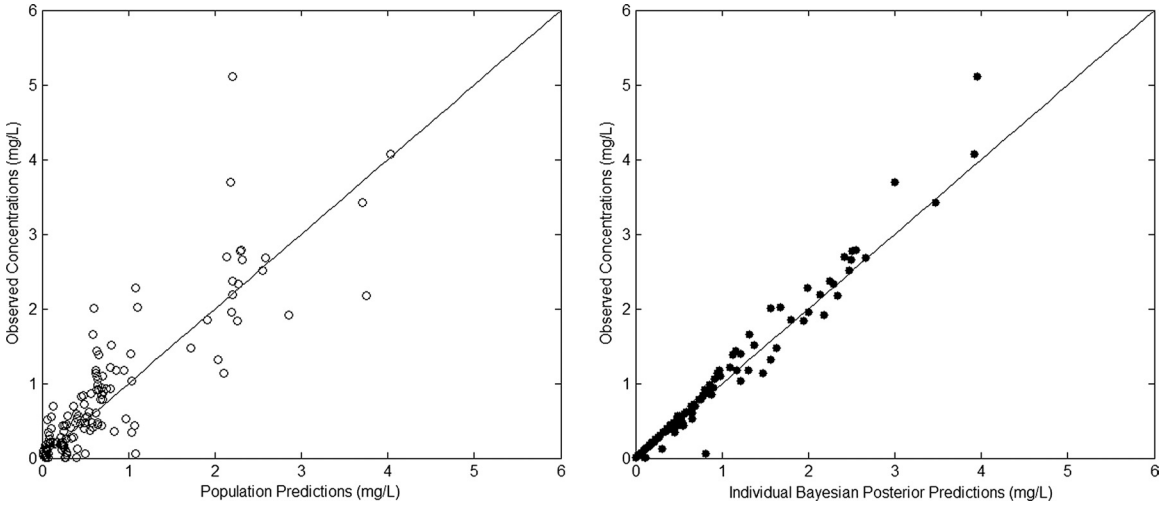

FIG 1 Observed atazanavir concentrations versus model predictions. (Left) Population predictions; (right) individual predictions based on Bayesian posterior parameters. The solid line is the line of identity ($y = x$).

sion equations as follows: population predictions, $y = 0.95x + 0.14$, $r^2 = 0.73$; individual predictions, $y = 1.08x - 0.03$, $r^2 = 0.97$. Mean errors of prediction were 0.10 ± 0.49 mg/liter and −0.04 ± 0.18 mg/liter, while the mean absolute errors of prediction were 0.31 ± 0.39 and 0.09 ± 0.15 mg/liter for population and individual predictions, respectively. The plot of weighted residuals versus time and the visual predictive check depicted in Fig. 2 and Fig. 3, respectively, also show acceptable model performance. Limited shrinkage was observed for model parameters, which indicated that data were sufficiently informative at the individual level (22). The percent shrinkage values estimated by the SAEM algorithm for random effects (η-shrinkage) were as follows: CL, 28% (regular absorbers) and 3% (low absorbers); V, 16% (regular absorbers) and 9% (low absorbers); $k_{a\ \mathrm{reg}}$, 31%; $k_{a\ \mathrm{low}}$, 23%.

Pharmacodynamic analysis. Individual pharmacokinetic parameters and various atazanavir exposure data are presented in Table 3. Patients who experienced virological failure had significantly lower absorption rate constant k_a ($P = 0.002$), AUC ($P = 0.001$), and AUC above the target concentration of 0.15 mg/liter ($P = 0.002$) than patients without failure. In addition, the proportion of low absorbers (100% versus 50%, $P = 0.014$) and the proportion of once daily atazanavir administration (86% versus 34%, $P = 0.012$) were significantly higher in patients who experienced virological failure than in patients without failure.

Results of the logistic regression analysis are summarized in Table 4. In the univariate analysis, four continuous variables, k_a, AUC_{0-24}, T_{target}, and C_{trough}, and one ordinal variable, the once-

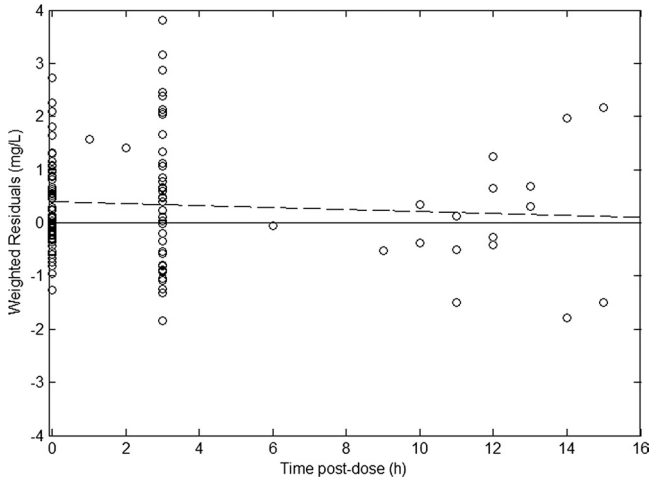

FIG 2 Weighted residuals versus time postdose. The dashed line is the regression line.

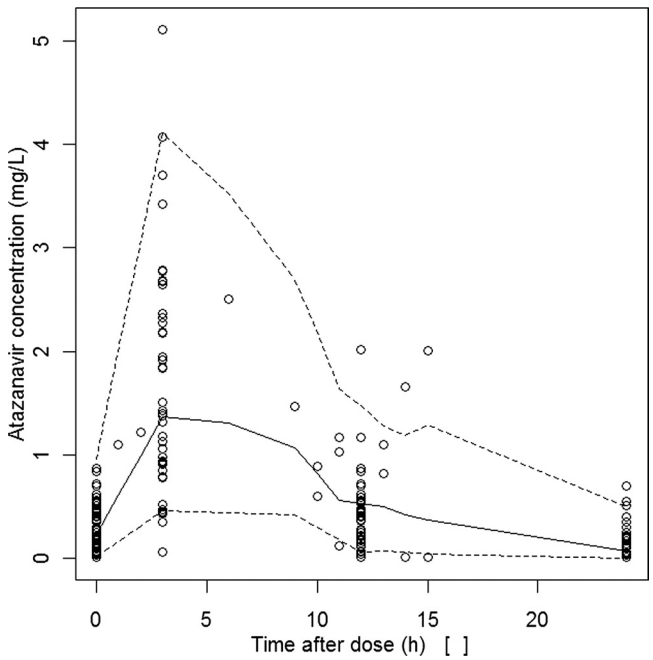

FIG 3 Visual predictive check from the final pharmacokinetic population model. Data points are the observed atazanavir concentrations; the solid line shows the median of simulated concentrations; dashed lines show 5th and 95th percentiles of simulated concentrations. The observed trough levels at 12 h (twice-daily regimen) and 24 h (once-daily regimen) postdose were duplicated for ease of graphical display.

TABLE 3 Comparison of pharmacokinetic parameter values and various exposure data in patients with and without virological failure

Parameter	No failure ($n = 62$)	Virological failure ($n = 7$)	P value[a]
CL (liters/h)	13.3 ± 2.6	15.3 ± 2.1	0.03
V (liters)	74.7 ± 24.6	81.5 ± 19.4	0.31
k_a (h^{-1})	0.75 ± 0.28	0.38 ± 0.20	0.002
AUC_{0-24} ($mg \cdot h \cdot liter^{-1}$)	22.4 ± 11.2	10.3 ± 2.1	0.001
AUC_{target} ($mg \cdot h \cdot liter^{-1}$)	18.9 ± 11.1	7.1 ± 1.9	0.002
T_{target} (h)	22.9 ± 2.5	19.9 ± 4.9	0.045
C_{trough} (mg/liter)[b]	0.31 ± 0.22	0.11 ± 0.08	0.012
No. (%) with $C_{trough} < 0.15$ mg/liter[b]	17 (27)	4 (57)	0.19
No. (%) of low absorbers	31 (50)	7 (100)	0.014
No. (%) receiving once-daily administration	21 (34)	6 (86)	0.012

[a] Mann-Whitney test (continuous variables) or Fisher exact test (ordinal variable).
[b] Individual trough concentration estimated from the pharmacokinetic model.

TABLE 4 Logistic regression analysis

Variable	OR[a]	95% CI	P value[b]
Univariate analysis			
CL	1.34	0.99–1.82	0.053
k_a	0.0034	4.9E-5-0.24	0.0008
AUC_{0-24}	0.66	0.43–1.016	0.0003
T_{target}	0.79	0.64–0.97	0.03
C_{trough}	3.0E-4	1.4E-7-0.63	0.005
$C_{trough} < 0.15$ mg/liter	3.53	0.71–17.44	0.12
Once-daily administration	11.7	1.3–103.8	0.007
Final multivariate model			
k_a	4.2E-4	10E-7-0.18	0.008
C_{trough}	5.6E-6	8.1E-11-0.39	0.005

[a] The odds ratio (OR) represents the variation of the probability of virological failure associated with a 1-unit increase of the independent variable. The ORs associated with a 0.1-unit change of the variable are 0.57 and 0.44 in the univariate models and 0.46 and 0.30 in the multivariate model, for k_a and C_{trough}, respectively.
[b] Likelihood ratio test, P value associated with the exclusion of each independent variable in the model.

daily administration, were identified as significant predictors of virological failure. In the subsequent multivariate analysis, only the absorption rate constant (k_a) and estimated C_{trough} significantly and independently influenced the probability of virological failure. For both variables, the probability of virological failure significantly increased when the value decreased. Of note, an alternative two-variable regression model, including k_a ($P = 0.0004$) and T_{target} ($P = 0.013$), was also identified, although the P value for the T_{target} variable fell short of statistical significance. The very low odds ratio values shown in Table 4 for the two variables of the final model might be considered surprising. However, it must be remembered that the odds ratio is the slope parameter in the logit model, thus representing the change in the probability of the outcome associated with a one-unit increase of the independent variable. Because a one-unit variation in the absorption rate constant or in ATV trough concentrations is a very large, unlikely interindividual variation (Table 3), one might consider instead the effect of a 0.1-unit variation in k_a and C_{trough} (see the footnotes to Table 4).

While the results of the logistic regression showed that ATV exposure was significantly associated with virological outcome, the final model had limited predictive ability, with an r^2 of 0.42, a positive predictive value of 60%, and a negative predictive value of 94%.

DISCUSSION

In this work, we used pharmacometric tools to analyze PK/PD data for unboosted atazanavir from a cohort of HIV-infected patients. A special feature of the final PK model is the mixture model used to describe ATV absorption. The pharmacokinetics of ATV is characterized by large interindividual variability, especially in the absorption process. Colombo et al. reported a mean absorption rate constant (k_a) of 0.41 h^{-1}, with an interindividual variability of 122% (expressed as coefficient of variation [CV]) (20). In another population PK study, Solas et al. found a mean k_a of 1.05 h^{-1}, with a CV of 156% (23). Other works have suggested that the pharmacokinetics of ATV should not be considered homogeneous and that mixture models may be a relevant approach to describe ATV pharmacokinetics. In an early study from ATV manufacturer, O'Mara et al. used a mixture model with two subpopulations for the absorption rate constant (k_a) and volume of distribution of the central compartment (11). In a recent study, a mixture model was also used to accommodate differences observed in ATV exposure between subjects, with two subpopulations for the absorption parameters k_a, lag time, and bioavailability (F) (21).

In our study, because data available in the absorptive phase were very sparse, the absorption parameter could not be accurately estimated. As a consequence, we put strong constraints on the absorption parameters, fixing the fixed effects for k_a, F, and lag time and setting no random effects for F and lag time. In spite of this limitation, the mixture model with two subpopulations for k_a and F greatly improved the fit. Interestingly, despite differences in study design, data, and final PK models, the proportions of high- and low-exposure subjects showed remarkable agreement across studies. We found an estimated 49% proportion of regular absorbers, while O'Mara et al. and Andrade et al. reported proportions of high exposure subjects of 48% and 51%, respectively (11, 21). This mixture model may be viewed as a surrogate to accommodate the influence of unobserved variables, such as compliance with drug treatment or food intake, on ATV exposure.

In the pharmacodynamic analysis, a significant relationship was found between ATV exposure and virological failure. Two pharmacokinetic indexes estimated from the PK model were associated with the outcome, the absorption rate constant k_a and ATV trough concentration. While this variable was not selected in the final logistic regression model, it is interesting that all patients who experienced virological failure were identified as low absorbers by the mixture PK model.

Several studies have reported a significant relationship between ATV exposure and virological response. In an early *in vitro* study, Drusano and colleagues found that time above threshold (time $>4\times$ EC_{50} [the viral 50% effective concentration]) was the pharmacodynamic variable linked with atazanavir antiviral effect (24). O'Mara and colleagues first reported a relationship between atazanavir AUC and the log drop in HIV load in a phase II study (11) Later, another unpublished study from the manufacturer reported a significant association between ATV C_{trough} and the probability of the HIV load being <400 as well as <50 copies/ml after 48 weeks of treatment in treatment-naive patients (25). Gonzalez de Requena and colleagues found a similar relationship be-

tween ATV C_{trough} and virological response at 12 weeks in ATV-naive patients, and they proposed a cutoff value of 0.15 mg/liter for ATV C_{trough} (17). Others groups used the genotypic inhibitory quotient (GIQ), defined as atazanavir trough concentration divided by the number of protease inhibitor-associated mutations, as a PK/PD index and reported a significant relationship between the GIQ and virological response (26–28). Reported cutoff values of GIQ were remarkably consistent across published studies, ranging from 183 to 230 ng/ml (0.183 to 0.230 mg/liter) per mutation.

The inhibitory quotient was not explored in this study, because data on PI-associated mutations were not available for all subjects in the cohort. Our results are in agreement with those of Bertz and Gonzalez de Requena (17, 25), although neither the suggested target C_{trough} of 0.15 mg/liter nor other indexes derived from it (i.e., AUC_{target} and T_{target}) were found to be significant predictors of virological response in the final model.

Efficacy was the main outcome of this cohort PK/PD study. However, a weak but significant positive correlation was found between estimated ATV C_{trough} and unconjugated bilirubin levels ($r^2 = 0.11$, $P = 0.006$; data not shown), as expected from previous reports (11, 17, 20).

Our study results also bring into question the optimal dosage regimen of uATV. Once-daily ATV administration was more frequent in patients who experienced virological failure than in patients without failure (Table 3). In addition, the estimated ATV C_{trough} values in patients who were administered uATV once daily (0.18 ± 0.17 mg/liter, $n = 27$) were lower than those in patients who received twice-daily uATV (0.36 ± 0.21 mg/liter, $n = 42$), a statistically significant difference ($P < 0.001$, Mann-Whitney test). This observation is in agreement with the results from a pharmacokinetic study which examined the consequence of switching from unboosted ATV at 400 mg once daily to 200 mg twice daily in 10 stable patients who were coadministered tenofovir and emtricitabine (29). The geometric means of ATV C_{trough} were 0.14 mg/liter and 0.31 mg/liter for the 400-mg once-daily and 200-mg twice-daily regimens, respectively ($P = 0.005$). Intracellular ATV trough concentrations also increased after the switch. As our study and others have identified ATV C_{trough} as a significant predictor of virological failure, twice-daily uATV administration appears to optimize drug exposure compared with the FDA-approved unboosted once-daily regimen. Although limited, available data indicate that average C_{trough} associated with the 200-mg twice-daily uATV regimen are much lower than the 0.85-mg/liter cutoff associated with hyperbilirubinemia (17), which suggests favorable tolerance. Further research is necessary to determine the optimal dosage design of uATV.

This study has several limitations that should be considered when the results are being interpreted, some of them being inherent to cohort studies. For example, food intake and compliance with drug treatment, which may significantly alter ATV exposure, could not be precisely assessed in such a study. Although the antiretroviral backbone was not controlled in this study, the vast majority of patients received similar antiretroviral regimens, based on two or more NRTIs in addition to uATV (Table 1). Important pharmacodynamic variables, such as susceptibilities of HIV isolates (EC_{50}) and binding to alpha-1 acid glycoprotein (24), were not available. As a consequence, a full mechanistic PK/PD modeling approach could not be carried out.

Data available for the pharmacokinetic analysis were relatively sparse. However, an adaptive model building was performed, and the final model parameters were estimated with acceptable precision. Also, the final model adequately described the data, with good predictive performance.

In conclusion, a population pharmacokinetic model, including a mixture model of absorption, adequately described ATV concentrations in a cohort of HIV-infected patients who were administered unboosted atazanavir. Significant differences in ATV pharmacokinetics and regimens have been observed between patients who experienced virological failure under uATV and those who did not. Atazanavir absorption rate constant and trough concentration significantly influenced the probability of virological failure in the logistic regression analysis. This work also suggests that twice daily administration of unboosted atazanavir may optimize drug therapy.

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REFERENCES

1. **Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, Cahn P, Eron JJ, Gunthard HF, Hammer SM, Reiss P, Richman DD, Rizzardini G, Thomas DL, Jacobsen DM, Volberding PA.** 2012. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society—U. S. A. panel. JAMA **308**:387–402.
2. **Daar ES, Tierney C, Fischl MA, Sax PE, Mollan K, Budhathoki C, Godfrey C, Jahed NC, Myers L, Katzenstein D, Farajallah A, Rooney JF, Pappa KA, Woodward WC, Patterson K, Bolivar H, Benson CA, Collier AC.** 2011. Atazanavir plus ritonavir or efavirenz as part of a 3-drug regimen for initial treatment of HIV-1. Ann. Intern. Med. **154**:445–456.
3. **Foca E, Ripamonti D, Motta D, Torti C.** 2012. Unboosted atazanavir for treatment of HIV infection: rationale and recommendations for use. Drugs **72**:1161–1173.
4. **Squires KE, Young B, Dejesus E, Bellos N, Murphy D, Zhao HH, Patel LG, Ross LL, Wannamaker PG, Shaefer MS.** 2010. Similar efficacy and tolerability of atazanavir compared with atazanavir/ritonavir, each with abacavir/lamivudine after initial suppression with abacavir/lamivudine plus ritonavir-boosted atazanavir in HIV-infected patients. AIDS **24**: 2019–2027.
5. **Ghosn J, Carosi G, Moreno S, Pokrovsky V, Lazzarin A, Pialoux G, Sanz-Moreno J, Balogh A, Vandeloise E, Biguenet S, Leleu G, Delfraissy JF.** 2010. Unboosted atazanavir-based therapy maintains control of HIV type-1 replication as effectively as a ritonavir-boosted regimen. Antivir. Ther. **15**:993–1002.
6. **European Medicines Agency.** 2012. Scientific discussion for the approval of Reyataz. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000494/WC500056377.pdf. Accessed 4 September 2012.
7. **Molto J, Santos JR, Valle M, Miranda C, Miranda J, Blanco A, Negredo E, Clotet B.** 2007. Monitoring atazanavir concentrations with boosted or unboosted regimens in HIV-infected patients in routine clinical practice. Ther. Drug Monit. **29**:648–651.
8. **Giuntini R, Martinelli C, Ricci E, Vichi F, Gianelli E, Madeddu G, Abeli C, Palvarini L, Penco G, Marconi P, Grosso C, Pellicano G, Bonfanti P, Quirino T.** 2010. Efficacy and safety of boosted and unboosted atazanavir-containing antiretroviral regimens in real life: results from a multicentre cohort study. HIV Med. **11**:40–45.
9. **Pavie J, Porcher R, Torti C, Medrano J, Castagna A, Valin N, Rusconi**

S, Ammassari A, Ghosn J, Delaugerre C, Molina JM. 2011. Efficacy and safety of a switch to unboosted atazanavir in combination with nucleoside analogues in HIV-1-infected patients with virological suppression under antiretroviral therapy. J. Antimicrob. Chemother. **66**:2372–2378.

10. **Hocqueloux L, Le Moal G, De la Tribonnière X, Prazuck T, Plainchamp D, Choisy P, Niang M, Mille C, Yazdanpanah Y, Parienti JJ.** 2011. Maintenance therapy with unboosted atazanavir is comparable to boosted strategy in experienced patients, even with tenofovir: a multicentre propensity-adjusted cohort, abstr. CDB359. Abstr. 6th IAS Conf. HIV Pathogenesis Treatment, Rome, Italy.
11. **O'Mara E, Cirincione B, Mummaneni V, Grasela T, Grasela D.** 2001. Population pharmacodynamic assessment of the safety and antiretroviral activity of atazanavir (BMS-232632), abstr. 507. Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., Chicago, IL.
12. **Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D.** 2000. A simplified equation to predict glomerular filtration rate from serum creatinine. J. Am. Soc. Nephrol. **11**:155A. (Abstract.)
13. **Beal SL.** 2001. Ways to fit a PK model with some data below the quantification limit. J. Pharmacokinet. Pharmacodyn. **28**:481–504.
14. **Bauer RJ.** 2009. NONMEM users guide. Introduction to NONMEM 7. ICON Development Solutions, Ellicott City MD.
15. Reference deleted.
16. **Yano Y, Beal SL, Sheiner LB.** 2001. Evaluating pharmacokinetic/pharmacodynamic models using the posterior predictive check. J. Pharmacokinet. Pharmacodyn. **28**:171–192.
17. **Gonzalez de Requena D, Bonora S, Canta F, Marrone R, D'Avolio A, Sciandra M, Milia MG, Di Garbo A, Sinicco A, Di Perri G.** 2005. Atazanavir C_{trough} is associated with efficacy and safety: definition of a therapeutic range, abstr. 645. Abstr. 12th Conf. Retrovir. Opportunistic Infect., Boston, MA.
18. **Buse A.** 1982. The likelihood ratio, Wald, and Lagrange multiplier tests: an expository note. Am. Stat. **36**:153–157.
19. **Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR.** 1996. A simulation study of the number of events per variable in logistic regression analysis. J. Clin. Epidemiol. **49**:1373–1379.
20. **Colombo S, Buclin T, Cavassini M, Decosterd LA, Telenti A, Biollaz J, Csajka C.** 2006. Population pharmacokinetics of atazanavir in patients with human immunodeficiency virus infection. Antimicrob. Agents Chemother. **50**:3801–3808.
21. **Andrade A, Baheti G, Smeaton L, Brundage RC, Kumarasamy N, Flanigan T, Hakim J, Braun K, Campbell T, Fletcher CV.** 2011. Sex- and geographic-based differences in atazanavir pharmacokinetics in subjects treated with didanosine, emtricitabine, and atazanavir in the AIDS clinical trials group (ACTG) prospective evaluation of antiretrovirals in resource limited settings (PEARLS) study, abstr. 648. Abstr. 18th Conf. Retrovir. Opportunistic Infect., Boston, MA.
22. **Savic RM, Karlsson MO.** 2009. Importance of shrinkage in empirical Bayes estimates for diagnostics: problems and solutions. AAPS J. **11**:558–569.
23. **Solas C, Gagnieu MC, Ravaux I, Drogoul MP, Lafeuillade A, Mokhtari S, Lacarelle B, Simon N.** 2008. Population pharmacokinetics of atazanavir in human immunodeficiency virus-infected patients. Ther. Drug Monit. **30**:670–673.
24. **Drusano GL, Bilello JA, Preston SL, O'Mara E, Kaul S, Schnittman S, Echols R.** 2001. Hollow-fiber unit evaluation of a new human immunodeficiency virus type 1 protease inhibitor, BMS-232632, for determination of the linked pharmacodynamic variable. J. Infect. Dis. **183**:1126–1129.
25. **Bertz R, Wang Y, Mahnke L, Persson A, Chung E, Mathew M, Agarwala S, Filoramo D, Hammond J, Grasela D.** 2007. Assessment of pharmacokinetic/pharmacodynamic relationships through 48 weeks from a study in HIV^+, ART-naive subjects receiving antiretroviral regimens containing atazanavir 400 mg or atazanavir/ritonavir 300/100 mg once daily, abstr. 565. Abstr. 14th Conf. Retrovir. Opportunistic Infect., Los Angeles, CA.
26. **Pellegrin I, Breilh D, Ragnaud JM, Boucher S, Neau D, Fleury H, Schrive MH, Saux MC, Pellegrin JL, Lazaro E, Vray M.** 2006. Virological responses to atazanavir-ritonavir-based regimens: resistance-substitutions score and pharmacokinetic parameters (Reyaphar study). Antivir. Ther. **11**:421–429.
27. **Cleijsen RM, van de Ende ME, Kroon FP, Lunel FV, Koopmans PP, Gras L, de Wolf F, Burger DM.** 2007. Therapeutic drug monitoring of the HIV protease inhibitor atazanavir in clinical practice. J. Antimicrob. Chemother. **60**:897–900.
28. **Solas C, Colson P, Ravaux I, Poizot-Martin I, Moreau J, Lacarelle B, Tamalet C.** 2008. The genotypic inhibitory quotient: a predictive factor of atazanavir response in HIV-1-infected treatment-experienced patients. J. Acquir. Immune Defic. Syndr. **48**:177–180.
29. **Bonora S, Gonzalez de Requena D, D'Avolio A, Calcagno A, Tettoni M, Siccardi M, Baietto L, Simiele M, Trentini L, Di Perri G.** 2011. Pharmacokinetics of switching unboosted atazanavir coadministered with tenofovir disoproxil fumarate from 400 mg once daily to 200 mg twice daily in HIV-positive patients. Antivir. Ther. **16**:499–504.